

# Impaired Cognitive Functioning in Patients with Tyrosinemia Type I Receiving Nitisinone

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**Objective** To examine cognitive functioning in patients with tyrosinemia type I treated with nitisinone and a protein-restricted diet.

**Study design** We performed a cross-sectional study to establish cognitive functioning in children with tyrosinemia type I compared with their unaffected siblings. Intelligence was measured using age-appropriate Wechsler Scales. To assess cognitive development over time, we retrieved sequential IQ scores in a single-center subset of patients. We also evaluated whether plasma phenylalanine and tyrosine levels during treatment was correlated with cognitive development.

**Results** Average total IQ score in 10 patients with tyrosinemia type I receiving nitisinone was significantly lower compared with their unaffected siblings ( $71 \pm 13$  vs  $91 \pm 13$ ;  $P = .008$ ). Both verbal and performance IQ subscores differed ( $77 \pm 14$  vs  $95 \pm 11$ ;  $P < .05$  and  $70 \pm 11$  vs  $87 \pm 15$ ;  $P < .05$ , respectively). Repeated IQ measurements in a single-center subset of 5 patients revealed a decline in average IQ score over time, from  $96 \pm 15$  to  $69 \pm 11$  ( $P < .001$ ). No significant association was found between IQ score and either plasma tyrosine or phenylalanine concentration.

**Conclusion** Patients with tyrosinemia type I treated with nitisinone are at risk for impaired cognitive function despite a protein-restricted diet. (*J Pediatr* 2014;164:398-401).

Hereditary tyrosinemia type I (OMIM 276700) is the most frequent inborn error of tyrosine degradation and results from a defect in fumarylacetoacetate hydrolase (EC 3.7.1.2). Without treatment, the accumulation of toxic metabolites, particularly maleylacetoacetate and fumarylacetoacetate, induces organ dysfunction and carcinogenesis. Patients may present within weeks of birth with gastrointestinal bleeding and liver failure, or later in childhood with failure to thrive, peripheral neuropathy, hepatic cirrhosis, and renal Fanconi syndrome.<sup>1</sup> Hepatocellular carcinoma is a frequent cause of death in early childhood.

Introduction of the drug 2-[2-nitro-4-trifluoromethylbenzoyl]-1, 3-cyclohexanedione (NTBC) in 1992 has dramatically improved the survival of patients with tyrosinemia type I.<sup>2,3</sup> NTBC, now marketed as nitisinone, prevents the accumulation of toxic metabolites by blocking an upstream enzyme, 4-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27), in the tyrosine degradation pathway.<sup>3</sup> Before the introduction of NTBC, the mortality rate in children diagnosed early (age <2 months) was 75% at age 2 years and >90% by age 12 years.<sup>4,5</sup> Long-term survival was attained only in those who underwent successful orthotopic liver transplantation after the discovery of hepatocellular carcinoma.<sup>6</sup> With the advent of NTBC, liver dysfunction is now controlled in >90% of patients, and extrahepatic manifestations have been abolished.<sup>7</sup> The risk of liver cancer has been reduced as well.<sup>8-10</sup> As a consequence, death in childhood has become a rare event.<sup>11</sup> Concerns whether increased survival is attained at the expense of reduced cognitive functioning remain, however.

Nitisinone biochemically switches the enzymatic defect from tyrosinemia type I to tyrosinemia type III, inducing elevated tyrosine concentrations up to  $1500 \mu\text{mol/L}$  (normal,  $40\text{--}90 \mu\text{mol/L}$ ) when dietary treatment is not provided.<sup>7</sup> Increased tyrosine levels are considered responsible for the impaired cognitive function in patients with tyrosinemia type II and III.<sup>1</sup> Whether a phenylalanine- and tyrosine-restricted diet aimed at reducing plasma tyrosine concentrations to  $<500 \mu\text{mol/L}$  is sufficient to prevent cognitive damage is unknown.

The aim of the present study was to evaluate cognitive functioning in patients with tyrosinemia type I during nitisinone treatment on a protein-restricted diet by comparing their IQ scores with those of their healthy siblings.

## Methods

Patients with tyrosinemia type I were retrieved from 2 Dutch centers, University Medical Center Utrecht and University Medical Center Groningen. Patients aged

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NTBC 2-[2-nitro-4-trifluoromethylbenzoyl]-1, 3-cyclohexanedione

>3 years who had ever been treated with nitisinone (at a dose of 0.8-2 mg/kg) and a protein-restricted diet were included. Exclusion criteria were lack of parental consent and failure to perform reliable IQ testing. Unaffected siblings, when available, served as controls. In the event that more siblings were eligible within the same family, the sibling in the same age category as the patient was asked to participate, to allow the use of the same IQ test and to optimize the comparison of the patient and control. A waiver of requirement for informed consent was granted by the Institutional Review Board of the University Medical Center Utrecht, which reviewed the study.

Cognitive functioning was assessed using age-appropriate Wechsler Scale IQ tests (Wechsler Preschool and Primary Scale of Intelligence-Revised for age 3-7 years, Wechsler Intelligence Scale for Children-Third Edition for age 7-17 years, and Wechsler Adult Intelligence Scale-Third Edition for age 18 years and older). These Wechsler tests provide 3 scores: a verbal IQ score, a performance IQ score, and a composite single total IQ score. The mean  $\pm$  SD score of these tests is  $100 \pm 15$ . Assessments took place at the participant's home, in a quiet room, during the daytime by a single investigator. The test results for the patients were compared with the results for the controls as well as with scores of IQ tests performed previously, when available.

Earlier studies have suggested that both tyrosine levels and phenylalanine levels may play a role in cognitive development.<sup>6,9</sup> To monitor the effect of dietary restriction, plasma amino acid concentrations, including phenylalanine and tyrosine, were determined at 3-month intervals during follow-up. These data were retrieved from patient records. Data on clinical manifestations during follow-up, particularly those suggestive of high levels of plasma tyrosine (particularly keratitis), were also collected from hospital records. Parental education, profession, and socioeconomic status were assessed using a questionnaire. The questionnaire also addressed siblings' school performance and health, including the use of medication.

Statistical analyses were performed using SPSS version 14.0 (SPSS, Chicago, Illinois). By defining the skewness ( $-0.04 \pm 0.524$ ), we found a normal distribution of the IQ scores and used the parametric Student *t* test to test for within-group IQ differences between patients and controls.

We used the paired Student *t* test to investigate the difference between paired patients and their unaffected siblings. We used Pearson correlation to examine relationships between performance on the IQ test and metabolic parameters in the patients with tyrosinemia type I. A significance level of  $P < .05$  was used for all tests.

## Results

Sixteen eligible patients with tyrosinemia type I were retrieved. Six patients were excluded, 4 patients because of lack of parental consent and 2 (3 years old) patients because reliable IQ testing could not be accomplished owing to significant developmental delay. Eight of the remaining 10 patients were treated with nitisinone and a protein-restricted diet at the time of testing, whereas 2 had received nitisinone for more than 10 years before undergoing liver transplantation (Table). In 7 families, an unaffected sibling served as a control.

### Cognitive Measures

The mean total IQ was 71 (range, 58-84) in patients with tyrosinemia type I and 91 (range, 78-104) in their healthy siblings. In patients with tyrosinemia, mean performance IQ was 70 (range, 59-81) and mean verbal IQ was 77 (range, 63-91) compared with 87 (range, 72-102) and 95 (range, 84-106) in their healthy siblings. The IQ difference was seen on both subscales and remained significant when only patients with an available sibling were analyzed (71 vs 91;  $P = .006$ ). The 2 patients who were no longer treated with nitisinone and a protein-restricted diet after undergoing liver transplantation had similarly low IQ levels. Low IQ scores were associated with special education attendance ( $r = -0.0677$ ;  $P = .036$ ).

In search of an explanation for these findings, we next focused on a subset of 5 patients from a single center. In this center, IQ tests were repeated at 2- to 3-year intervals as a regular part of follow-up. We reasoned that a stable IQ over time would point toward preexisting factors, whereas a decline in IQ would suggest ongoing damage during—and perhaps due to—treatment. As depicted in Figure 1, initial IQs were within the normal range in 4 of the 5

**Table.** Baseline characteristics of patients with tyrosinemia type I

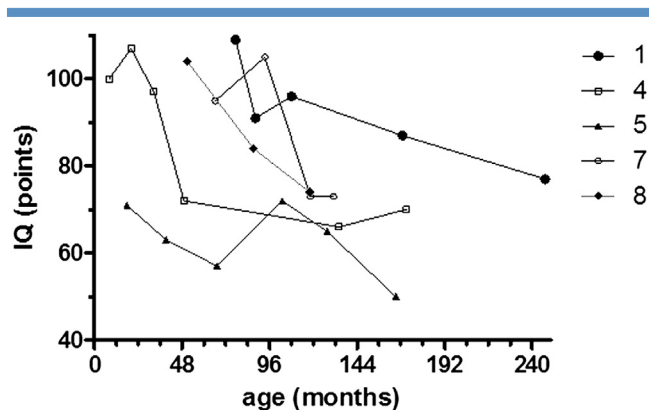
Patient	Age at time of study, y	Sex	Age at diagnosis, mo	Age at start of nitisinone use, mo	Current NTBC use	Liver transplantation	History of keratitis	Unaffected sibling included	Education	SES
1*	20	Male	3	78	Yes	No	Yes	Yes	Regular	Low
2	20	Female	9	72	No†	Yes	No	Yes	Regular	Low
3	16	Male	6	6	Yes	No	Yes	Yes	Regular	Middle
4	14	Male	8	8	Yes	No	Yes	Yes	Regular	Middle
5	13	Male	3	3	Yes	No	Yes	Yes	Special	Low
6	13	Male	24	24	No‡	Yes	No	No	Special	Middle
7*	11	Male	0	0	Yes	No	Yes	Yes	Regular	Low
8	10	Male	6	6	Yes	No	Yes	Yes	Special	Low
9	9	Male	4	4	Yes	No	No	Yes	Regular	High
10	5	Female	3	3	Yes	No	No	No	Regular	Middle

SES, socioeconomic status.

\*Patients 1 and 7 are siblings.

†At age 18 y.

‡At age 8 y.



**Figure 1.** Longitudinal IQ levels in patients with tyrosinemia type I receiving nitisinone. A significant drop of total IQ (an average of 27 IQ points;  $P < .001$ ) can be seen.

patients, but a significant decline in their total IQ scores was observed during follow-up. The average total IQ dropped from  $96 \pm 15$  to  $69 \pm 11$  between each patient's first and last measurement, a decline of 27 IQ points ( $P < .001$ ).

### IQ and Metabolic Control

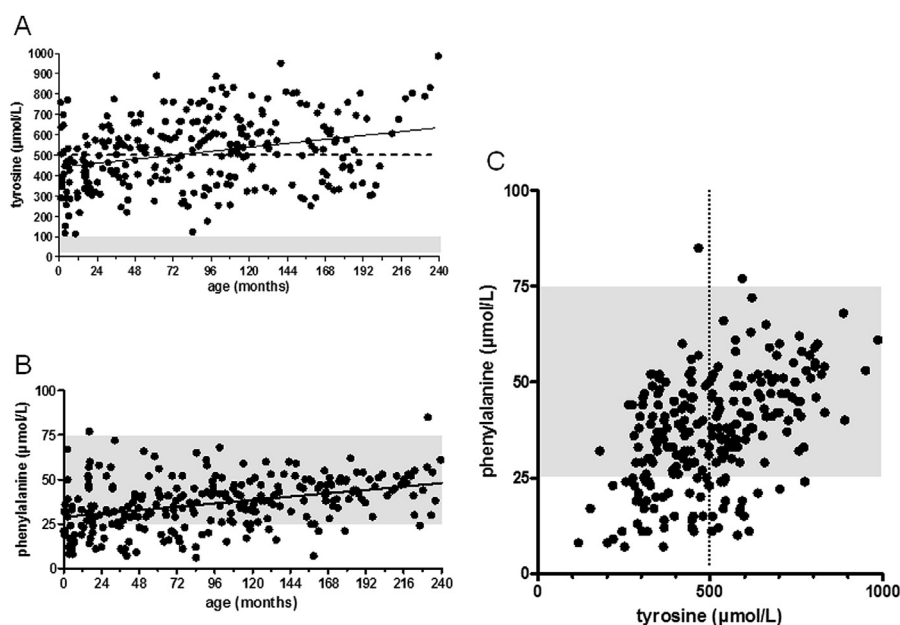
To investigate the relationship between IQ and the degree of metabolic control, we analyzed plasma tyrosine and phenylalanine levels in the 8 patients treated with nitisinone and a protein-restricted diet (Figure 2). Plasma tyrosine was elevated in all patients (mean,  $511 \pm 168 \mu\text{mol/L}$ ). Although the mean plasma phenylalanine level was within the normal

range (mean,  $37 \pm 14 \mu\text{mol/L}$ ), a substantial proportion (24%) of measured values were below the lower limit of the normal range ( $25 \mu\text{mol/L}$ ). A significant correlation was found between tyrosine levels and phenylalanine levels ( $r = 0.411$ ;  $P = .001$ ) (Figure 2, A). Both tyrosine and phenylalanine levels increased with age ( $r = 0.087$ ;  $P < .001$  and  $r = 0.144$ ;  $P < .001$ , respectively). Neither plasma tyrosine nor phenylalanine level was correlated with IQ indices.

## Discussion

Our findings show that patients with tyrosinemia type I treated with nitisinone are at risk for developing impaired cognitive function despite a protein-restricted diet. Treating patients with tyrosinemia type I with a combination of nitisinone and a protein-restricted diet has brought about improvements in both life expectancy and disease-related morbidity. Consequently, patients with tyrosinemia type I are now likely to reach adulthood. This makes the major finding reported here—an average IQ of 71, 20 IQ points lower than their unaffected siblings—of both clinical and social relevance.

To control for genetic and/or environmental background, we compared IQ scores in our patients and their siblings. The somewhat lower average IQ in healthy siblings compared with the general population supports the usefulness of this approach. Notwithstanding, the IQ of the patients was substantially lower than that of their siblings. These patients' poor school performance corroborates a previous finding reported by Masurel-Paulet et al<sup>13</sup> and underscores that the



**Figure 2.** Tyrosine and phenylalanine plasma concentrations in patients with tyrosinemia type I receiving nitisinone. Gray areas indicate reference values. The dashed line indicates the therapeutic target. **A**, Plasma tyrosine level by age. **B**, Plasma phenylalanine levels by age. **C**, Correlation between plasma tyrosine and phenylalanine levels.

significantly lower IQ is of clinical relevance. One of the main strengths of the present study is the actual availability of IQ levels, including those over time. IQ testing in this center was not performed on a “clinical suspicion” basis, which obviously would have represented a selection bias, but rather was performed routinely in all patients based on initial concerns regarding the long-term cognitive effects of nitisinone.

The nature of the observed cognitive impairment remains enigmatic. Mild impaired cognitive function may have been a “missed” feature of tyrosinemia type I, a symptom that we failed to recognize before the use of nitisinone because of the short life span of untreated patients with tyrosinemia type I. Unfortunately, there are no animal data known to us to either support or refute this hypothesis. Nonetheless, we are aware of adults with tyrosinemia and normal cognitive function.<sup>14</sup> The lower IQ may be an unwanted side effect of treatment. In support, a significant decrease in IQ was noted in a subset of patients in whom IQ was regularly tested. The finding of similarly low IQs in patients who had stopped taking nitisinone after undergoing liver transplantation argues against the acute toxicity of nitisinone itself. Most likely, nitisinone affects cognitive function indirectly, by inducing profoundly elevated plasma tyrosine levels. The lack of correlation between IQ and tyrosine levels in our population may be related to the limited sample size or other issues with regard to tyrosine measurement.

Tyrosine levels can be reduced through a more restricted protein intake; however, this measure may be detrimental if it induces lower phenylalanine levels. Because phenylalanine and tyrosine compete for transport to the brain, the combination of high tyrosine and low phenylalanine may lead to insufficient phenylalanine transport to the brain, decreasing the amount of phenylalanine available for protein and neurotransmitter synthesis. According to some authors, this shortage may cause a deviant cognitive development that can be expressed later in life with mildly impaired cognitive function.<sup>12,13,15</sup> Hopefully, data on the effects of a more restrictive diet will become available. Based on our data, we recommend that the follow-up of patients with tyrosinemia type I include routine cognitive assessment. ■

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## References

1. Mitchell GA, Grompe M, Lambert M, Tanguay RM. Hypertyrosinemia. In: Scriver CR, Beaudet AL, Sly WS, et al., eds. *The metabolic and molecular bases of inherited disease*. New York: McGraw-Hill; 2001. p. 1777-82.
2. Lindstedt S, Holme E, Lock EA, Hjalmarson O, Strandvik B. Treatment of hereditary tyrosinemia type I by inhibition of 4-hydroxyphenylpyruvate dioxygenase. *Lancet* 1992;340:813-7.
3. Russo PA, Mitchell GA, Tanguay RM. Tyrosinemia: a review. *Pediatr Dev Pathol* 2001;4:212-21.
4. Scott CR. The genetic tyrosinemias. *Am J Med Genet C Semin Med Genet* 2006;142C:121-6.
5. van Spronsen FJ, Thomasse Y, Smit GP, Leonard JV, Clayton PT, Fidler V, et al. Hereditary tyrosinemia type I: a new clinical classification with difference in prognosis on dietary treatment. *Hepatology* 1994;20:1187-91.
6. Wijburg FA, Reitsma WCC, Slooff MJH, van Spronsen FJ, Koetse HA, Reijngoud DJ, et al. Liver transplantation in tyrosinemia type I: the Groningen experience. *J Inher Metab Dis* 1995;18:114-8.
7. McKiernan PJ. Nitisinone in the treatment of hereditary tyrosinemia type I. *Drugs* 2006;66:743-50.
8. Holme E, Lindstedt S. Nontransplant treatment of tyrosinemia. *Clin Liver Dis* 2000;4:805-14.
9. van Spronsen FJ, Bijleveld CM, van Maldegem BT, Wijburg FA. Hepatocellular carcinoma in hereditary tyrosinemia type I despite 2-(2-nitro-4,3-trifluoro- methylbenzoyl)-1, 3-cyclohexanedione treatment. *J Pediatr Gastroenterol Nutr* 2005;40:90-3.
10. Koelink CJ, van Hasselt P, van der Ploeg A, van den Heuvel-Eibrink MM, Wijburg FA, Bijleveld CM, et al. Tyrosinemia type I treated by NTBC: how does AFP predict liver cancer? *Mol Genet Metab* 2006;89:310-5.
11. Holme E, Lindstedt S. Tyrosinemia type I and NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1, 3-cyclohexanedione). *J Inher Metab Dis* 1998;21:507-17.
12. Wilson CJ, van Wyk KG, Leonard JV, Clayton PT. Phenylalanine supplementation improves the phenylalanine profile in tyrosinemia. *J Inher Metab Dis* 2000;23:677-83.
13. Masurel-Paulet A, Poggi-Bach J, Rolland MO, Bernard O, Guffon N, Dobbelaere D, et al. NTBC treatment in tyrosinemia type I: long-term outcome in French patients. *J Inher Metab Dis* 2008;31:81-7.
14. Kim SZ, Kupke KG, Ierardi-Curto L, Holme E, Greter J, Tanguay RM, et al. Hepatocellular carcinoma despite long-term survival in chronic tyrosinaemia I. *J Inher Metab Dis* 2000;23:791-804.
15. van Spronsen FJ, Smit GPA, Wijburg FA, Thomasse Y, Visser G, Heymans HSA. Tyrosinemia type I: consideration of treatment strategy and experience with risk assessment, diet and transplantation. *J Inher Metab Dis* 1998;18:111-4.